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Amylose, Tocopherol, Free Sugar and Fatty Acid Content in Selected Mutant Lines of *Oryza sativa* cv. Shindongjin

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Abstract

To assess the potential as biofortified rice varieties, new endosperm and grain mutant lines were selected from M_4 generation seeds of the rice cultivar Shindongjin, which were either γ-irradiated or treated with *N*-methyl-*N*-nitrosourea (MNU) and lipid, sugar, and tocopherol content were analyzed. Amylose content in non-waxy mutants ranged from 8.8% in SM-4, a dull-type mutant, to 29.5% in SM-51, compared to 18.9% in the parental variety, Shindongjin. SM-23, a floury-type mutant, contained 0.09 μg/g α-tocopherol (40.9% of total tocopherol), was three times higher than in the parental variety. SM-32, a giant embryo-type mutant, had a 2.2-fold higher total tocopherol content, 2.1-fold higher α-tocopherol, and 5.5-fold higher δ-tocopherol content (47.3% of total tocopherol) than the parental variety (0.13 μg/g). Total free sugar content was elevated in all selected mutants and 1.2-8.6 times higher than in the parental variety (11.38 μg/g). These increased sugar levels were due to increase in sucrose concentration. SM-23 (floury-type mutant) and SM-51 (high amylose-type mutant) had 4.6 and 7.0 times more sugar, respectively, than the parental variety (11.38 μg/g). With relatively high concentrations, most mutants showed elevated fatty acid content in the SM 32 (giant embryo-type) and SM-51 (high amylose-type) mutants, at 124.56 and 89.59 mg/g, respectively. All selected mutants displayed valuable characteristics for the development of new varieties in rice-breeding programs.

Key words: amylose, endosperm mutant, fatty acid, free sugar, γ-irradiation, *N*-methyl-*N*-nitrosourea, tocopherol

Introduction

Although daily rice consumption is decreasing in Korea due to changes in eating habits and lifestyles, the demand for high quality and new biofortified rice varieties is increasing in worldwide. For example, tocopherol content is an important trait in rice because of its role in human health. Tocopherol is a phenolic compound with an isoprenoid side chain attached to an aromatic ring of the chromanol moiety. It is classified into tocopherol and tocotrienol based on the presence of a double bond on the side chain and α-, β-, γ-, and δ-tocopherol are differentiated by the methyl groups on the chromanol ring (Bourgeois 1992; Qureshi et al. 2000). Tocopherol is fat-soluble and inhibits lipid oxidation in human blood, tissue, and cells, thereby lowering blood pressure and preventing arteriosclerosis (Blankenhorn and Clewing 1993). The antioxidative effect of particle-bounded tocopherol increases in the order $\alpha<\beta<\gamma<\delta$ (Niki et al. 1986). In addition, the antioxidative activities of γ- and δ-tocopherol are superior to α-tocopherol under different temperature and substrate conditions (Ikeda and Fukuzumi 1977; Koskas et al. 1984).

The sugar content in brown rice is normally 0.7-1.3% (Juliano 1985a) with fructose, glucose, and sucrose as the most common free sugars. In sensory tests, the correlation between total sugar content and taste value has been found to be low (Chikubu et al. 1983). However, other studies have reported the

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correlation between fructose/glucose and taste value (Chida and Tajima 2004; Maruyama et al. 1983). Sugar analysis in each rice particle layer has shown that the surface layer of white rice has strong taste elements (Kano and Tajima 2001; Maruyama et al. 1983). Poly- and oligosaccharides have been purified from these elements by column chromatography. The oligosaccharides were contained in the outer embryo layer (sapio-layer) of water-extracted and steamed rice, as well as in high-quality rice (Chida and Tajima 2004; Tajima et al. 1992). High-viscosity polysaccharides have also been found recently in water-extracted starch granules (Tajima et al. 1992). During cooking, these polysaccharides are released, coating the steamed rice grains and raising viscosity. This may be an important indicator of rice quality and sensory values as the aggregation of the polysaccharides with free amino acids and vitamins (Tajima et al. 1992).

Lipids including fatty acids and glycerol are divided into saturated and unsaturated fatty acids (Choudhury and Juliano 1980; Fujino 1978). The total lipid content of brown and white rice grains is 2-3 and 0.8-1.3%, respectively, and there is no difference between Indica and Japonica varieties (Mano et al. 1999). In brown rice, 22, 56, and 22% of total lipid content are distributed in the aleurone layer, subaleurone layer, and embryo, respectively and lipid bodies are 0.7-3 μm in size within the cell (Godber and Juliano 2003). Starch- and non-starch-bound lipids, in particular, comprise 0.5-1.0% and 0.4% of total lipid content (w/w) in white rice. The principle non-starch-bound lipids are linoleic, oleic, and palmitic acids (Hemavathy and Prabhakar 1987; Taira et al. 1988), which are destroyed during cooking (Begum et al. 2000). Starch-bound lipids are mainly mono-acyl lipids (fatty acids and phospholipids) and lipids, such as palmitic and linoleic acid are bound with amylose affect on cooking characteristics and modify the starch and rice grain (Kaur and Singh 2000). When rice cells are damaged by physical or biological factors, lipids are hydrolyzed by lipase and convert to glycerol and fatty acids. The free fatty acids combine with the spiral structure of starch, which inhibits "pasting" during cooking and hardening of the cooked rice. During storage, the lipids in rice degrade faster than starch or protein. So, the amount of KOH (in mg) required for neutralization of fatty acids in 100 g of rice powder has been used to estimate the degree of fatty acids oxidation as the indicator of quality deterioration in rice (Shibuya et al. 1974). A higher oleic acid/linoleic acid content leads to more stable products during storage. Lipid oxidation combined with protein denaturation affects not only the stickiness of cooked rice and taste quality but also senescence (Hibi et al. 1990). Nutritional quality can be improved by conventional and molecular breeding techniques, however, variation in rice germplasm is crucial for breeding programs. Mutation breeding is one way to create genetic variation. In the present study, the Japonica rice variety Shindongjin was mutated using γ-irradiation and *N*-methyl-*N*-nitrosourea (MNU) treatment to induce variations in free sugar content and other quality traits.

Materials and Methods

Plant materials

Seeds of embryo mutant lines induced by γ-irradiation and MNU were sown, with Shindongjin as a control, on April 20, 2006 and transplanted on May 30. Planting density was 50×30 cm, as a single plant per hill and 15 plants per line, with three replicates. Fertilizer was applied at a rate of 9:4:5 kg (N:P:K) per 10 ha. A 1000-grain weight of harvested rice grains was estimated after drying in the shade and threshing. Brown rice was prepared using a huller (Ssanyong, SY88-TH), ground to a fine powder, and filtered through an 80-mm mesh prior to analysis. All images were recorded using a digital camera. Embryo structures were observed under a stereomicroscope (Leica MZ16).

Amylose content

Amylose content was estimated by colorimetric analysis (Juliano 1985a). Briefly, a paste was mixed for 10 min in a 200-ml flask using 100 mg of brown rice in boiling water combined with 1 ml of 95% ethanol and 9 ml of 1 N NaOH. After cooling to room temperature, 100 ml of distilled water were added and a 5-ml aliquot was transferred to a new flask. Then, 1 ml of 1 N acetic acid and 2 ml of 2% I_2-KI solution were added and the volume was topped up to 100 ml with distilled water. Absorbance was measured at 620 nm using a spectrophotometer.

Tocopherol analysis

A 0.5-g sample of rice power was suspended in 20 ml of extraction solvent (methanol/ethyl acetate/petroleum ether; MeOH/EtOAC/P-Ether, 1:1:1, v/v) and 1 ml of internal standard (α-tocopherol acetate) solution was added and mixed for 30 s. After cooling for 10 min at room temperature, extracts were filtered three times through Whatman No. 4 filter paper and concentrated to 60 ml. Then, 30 ml of ethyl acetate and 1.5 ml of saturated KOH were added to the filtrate and incubated for 16 h and separately extracted twice with distilled water at the 1:2 ratio. The ethyl acetate layer was collected, filtered through No. 4 filter paper, and dissolved in 2 ml methanol (1% butylated hydroxy-toluene, BHT). After filtration through a 0.45-μm filter, the sample was analyzed by high-performance liquid chromatography (HPLC; Shimadzu 10A) under optimized conditions; a Supelco C_{18}, 250×4.6-mm column (5 μm particles), elution with 90% methanol, flow-rate 1.6 ml/min, 20 μl injection volume, and UV at 215 nm on a photodiode array detector (Ryynänen et al. 2004).

Free sugar analysis

A 0.5-g sample of rice flour was sonicated in 6 ml of 75% ethanol for 1 h and filtered through No. 4 filter paper and a 0.45-μm syringe filter. The filtrate was analyzed by HPLC (Shimadzu 10A) under optimized conditions; a Supelco C_{18}, 250×4.6 mm column (5 μm particles), elution with solution A (acetonitrile)

Table 1. Amylose content and 1000-grain weight of selected endosperm type and grain mutants.

Number of mutant line	Character of endosperm	Amylose content (%)	1000-grain weight (g)
Wild type (Shindongjin)	Normal	18.9^{bcf}	27.73^{bc}
SM-01$^{(M)}$	Waxy	4.3^{e}	26.67^{bc}
SM-04$^{(\gamma)}$	Dull	8.8^{d}	25.33^{bc}
SM-09$^{(\gamma)}$	Chalky	21.7^{bc}	26.67^{bcd}
SM-10$^{(M)}$	Chalky	17.2^{bc}	23.00^{bcd}
SM-21$^{(\gamma)}$	Floury	22.6^{b}	24.00^{cde}
SM-23$^{(\gamma)}$	Floury (wrinkled)	19.4^{b}	20.33^{cde}
SM-26$^{(\gamma)}$	Shrunken	16.7^{c}	18.00^{ef}
SM-31$^{(\gamma)}$	Sugary	17.7^{bc}	13.00^{f}
SM-32$^{(\gamma)}$	Giant embryo	21.4^{bc}	25.33^{bcde}
SM-35$^{(\gamma)}$	White core	20.4^{bc}	25.67^{bc}
SM-51$^{(\gamma)}$	High amylose	29.5^{a}	18.67^{ef}
Mean		18.2	22.86
F-value		23.74*	7.08*

* Significant at p=0.01.

† Superscripted different letters within the same columns indicate a significant difference.

(M): Lines induced by *N*-methyl-n-nitrosourea (MNU).

(γ): Lines induced by γ-irradiation.

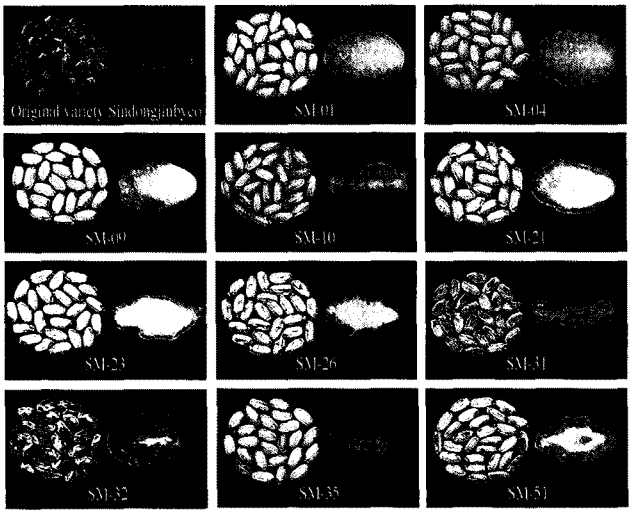

Fig. 1. Cross-sectional stereomicrographs of whole seeds (brown) of selected endosperm and grain-type mutants. SM-01^{M}): waxy; SM-04$^{\gamma}$): dull; SM-09$^{\gamma}$): chalky; SM-10^{M}): chalky; SM-21$^{\gamma}$): floury; SM-23$^{\gamma}$): floury (winkled); SM-26$^{\gamma}$): shrunken; SM-31$^{\gamma}$): sugary; SM-32$^{\gamma}$): giant embryo; SM-35$^{\gamma}$): white core; SM-51$^{\gamma}$): high amylose. (M): Lines induced by MNU; (γ): Lines induced by γ-irradiation.

and solution B (0.04% NH_4OH), flow-rate 1.0 ml/min, gradient elution with 75:25 (A/B) for 30 min, 20 µl injection volume, and UV at 215 nm on a photodiode array detector. Peak area was calculated and represented as relative percentage (Gnansounou et al. 2005).

Fatty acid analysis

Fatty acids were extracted from rice flour (0.5 g), as described by Folch et al. (1957). Pentadecanoic acid (PDA) was used as the internal standard. Standards for free acids and methyl esters were purchased from Sigma (St. Louis, MO, USA) and dissolved in methanol prior to use.

Extracted fatty acids were methylesterized using the methods of Chung (1991) and Ruibal-Mendieta et al. (2004). They were then subjected to gas chromatography (GC) on a Hewlett Packard 5890 (series II) using an HP-20M (0.2 mm, 25 m, 0.1 µm) column, 180°C (injector 200°C, detector 210°C), flame ionization detector (FID) detector, and a nitrogen gas carrier. For the purification and quantification of heavy chain fatty acids, modified conditions of an HP-5 column (0.2 mm, 25 m, 33 µm), column temperature program (158°C, 3 min, 5°C/min, 190°C) were applied. For extraction and methylation, a freeze-dried sample of rice flour (0.3 g) was extracted by sonication with 5 ml of chloroform/methanol (2:1, v/v) and PDA (1 mg) as an internal standard. After addition of 5 ml of 0.58% NaCl, the extract was centrifuged at 200 rpm for 10 min. The chloroform layer was concentrated using N_2 gas and hydrolyzed with 2 ml of toluene and 0.5 ml of 2 N NaOH-methanol in boiling water for 3 min. After cooling at room temperature, methylation was performed with 2.5 ml of 14% BF_3 for 5 min in boiling water and 15 ml of dH_2O and 10 ml of petroleum ether were added to the reaction mixture. After cooling at room temperature for 10 min agian, the petroleum ether layer was dried with sodium sulfate (Kim et al. 1995; Metcalfe et al. 1966).

Results and Discussion

Images of endosperm mutants derived from the M_4 generation were shown in Fig. 1. Amylose content and the 1000-grain weight of endosperm mutants were shown in Table 1. The amylose content in non-waxy mutants ranged from 8.8% in SM-4, a dull-type mutant, to 29.5% in SM-51, compared to 18.9% in the parental variety, Shindongjin. All selected endosperm mutants showed lower 1000-grain weight than the parent variety. The 1000-grain weight of the SM-31 (a sugary-type mutant) and SM-26 (a shrunken type), in particular, were 13 and 18 g, respectively, i.e., 2.1 and 1.5 times lower than the parental variety (27.73 g), respectively. These two lines presented an intensively crushed grain shape (Fig. 1).

Tocopherol analysis

Tocopherol content of the endosperm mutants was shown in Table 2. SM-23, a floury-type mutant, contained 0.09 µg/g α-tocopherol (40.9% of total tocopherol), which was 3-fold higher than the parental variety (0.03 µg/g). SM-32 (a giant-embryo-type) had 0.27 µg/g in total tocopherol with 0.07 µg/g α-tocopherol and 0.13 µg/g δ-tocopherol (47.3% of total tocopherol), which was 2.2 times more total tocopherol, 2.1 times more α-tocopherol, and 5.5 times more δ-tocopherol than the parental

Table 2. Tocopherol content in the different endosperm type and grain mutants.

Number of mutant line	Character of endosperm	Rice tocopherol				Total[(1)]
		α-	β-	γ-	δ-	
		μg/g (%)				
Parental variety (Shindongjin)	Normal	0.03[de†] (23.0)	0.04 (30.8)	0.04 (30.8)	0.02 (15.4)	0.13
SM-01[(M)]	Waxy	0.03[e] (21.4)	0.03 (21.4)	0.05 (35.8)	0.03 (21.4)	0.14
SM-04[(γ)]	Dull	0.03[e] (20.0)	0.04 (26.7)	0.05 (33.3)	0.03 (20.0)	0.15
SM-09[(γ)]	Chalky	0.07[abcd] (41.2)	0.03 (17.6)	0.04 (23.5)	0.03 (17.6)	0.17
SM-10[(M)]	Chalky	0.07[abc] (38.9)	0.02 (11.1)	0.06 (33.3)	0.03 (16.7)	0.18
SM-21[(γ)]	Floury	0.06[abcde] (35.3)	0.02 (11.8)	0.07 (41.2)	0.02 (11.8)	0.17
SM-23[(γ)]	Floury (wrinkled)	0.09[a] (40.9)	0.03 (13.6)	0.07 (31.9)	0.03 (13.6)	0.22
SM-26[(γ)]	Shrunken	0.07[abc] (35.0)	0.04 (20.0)	0.07 (35.0)	0.02 (10.0)	0.20
SM-31[(γ)]	Sugary	0.08[ab] (40.0)	0.04 (20.0)	0.04 (20.0)	0.04 (20.0)	0.20
SM-32[(γ)]	Giant embryo	0.07[abc] (25.9)	0.04 (14.8)	0.03 (11.1)	0.13 (48.2)	0.27
SM-35[(γ)]	White core	0.04[cde] (33.3)	0.02 (16.7)	0.03 (25.0)	0.03 (35.0)	0.12
SM-51[(γ)]	High amylose	0.06[abcde] (33.3)	0.04 (22.2)	0.04 (22.2)	0.04 (22.2)	0.18
Mean		0.06	0.03	0.05	0.04	0.18
F-value		3.61*	0.9	1.08	1.07	1.17

* Significant at p=0.01.

† Superscripted different letters within the same columns indicate a significant difference.

α-: α-tocopherol; β-: β-tocopherol; γ-: γ-tocopherol; δ-: δ-tocopherol.

(1): α-tocopherol+β-tocopherol+γ-tocopherol+δ-tocopherol.

(M): Lines induced by MNU.

(γ): Lines induced by γ-irradiation.

Table 3. Composition and content of free sugars in the different endosperm type and grain mutants.

Number of mutant line	Character of endosperm	Fructose	Glucose	Sucrose	Total[(1)]
		mg/g (%)			
Wild type (Shindongjin)	Normal	0.18 (1.6)	0.29 (2.5)	10.92 (96.0)	11.38
SM-01[(M)]	Waxy	0.19 (1.4)	0.19 (1.4)	13.41 (97.2)	13.79
SM-04[(γ)]	Dull	0.21 (1.6)	0.15 (1.1)	12.75 (97.3)	13.11
SM-09[(γ)]	Chalky	0.25 (1.3)	0.26 (1.4)	18.21 (97.3)	18.72
SM-10[(M)]	Chalky	0.17 (0.9)	0.21 (1.2)	17.82 (97.9)	18.20
SM-21[(γ)]	Floury	0.21 (0.9)	0.37 (1.7)	21.81 (97.4)	22.40
SM-23[(γ)]	Floury (wrinkled)	0.55 (1.1)	1.35 (2.6)	50.19 (96.4)	52.09
SM-26[(γ)]	Shrunken	0.49 (0.9)	0.98 (1.7)	55.30 (97.4)	56.77
SM-31[(γ)]	Sugary	4.06 (4.2)	6.50 (6.7)	86.95 (89.2)	97.51
SM-32[(γ)]	Giant embryo	0.26 (1.5)	0.18 (1.0)	16.77 (97.4)	17.21
SM-35[(γ)]	White core	0.17 (0.9)	0.21 (1.1)	18.00 (97.9)	18.38
SM-51[(γ)]	High Amylose	3.20 (4.2)	5.89 (7.7)	67.00 (88.1)	76.09
Mean		1.11	2.09	31.63	34.84

(1): Fructose+Glucose+Sucrose.

(M): Lines induced by MNU.

(γ): Lines induced by γ-irradiation.

variety. These results were commercially valuable as health-care products because cosmetics containing tocopherol and tocotrienol from rice bran, such as "MaxLife Rice Tocotrienols" and "NutriRice," have been already marketed in the USA and Japan. Total tocopherol content showed a positive correlation with α-tocopherol (r=0.697) and δ-tocopherol (r=0.762) at the 1% level. Total fatty acid content was positively correlated with total tocopherol content (r=0.696), particularly with δ-tocopherol content(r=0.774). The α-tocopherol and γ-tocopherol contents of opaque, shrunken, floury, and sugary-type mutants were 2-3 and 1.5-2 times greater than that of the parental variety, respectively. This positive correlation between total fatty acid and total tocopherol content will be valuable in future breeding programs to improve the tocopherol content of rice varieties.

Free sugar analysis

Rice-breeding programs aim to diversify the rice germplasm with improved quality and industrial usage (Heu and Park 1990). Rice mutants with varying amylose content, modified starch storage tissue, and increased sugar content have been developed by artificial mutagenesis (Kim et al. 1991; Okuno and Yano 1984; Satoh and Omura 1981). The free sugar content and grain shape of the endosperm rice mutants were analyzed to confirm the relationship between mutant types and sugar content. All mutants showed a 1.2-8.6 times higher sugar content than the parental variety, with the sucrose ration being relatively higher in all mutants (Table 3). Four mutants (SM-23, SM-26, SM-31, and SM-51) showed high level of total sugar content (52.09, 56.77, 97.51, and 76.01 mg/g, respectively). The sugar composition of SM-23 and SM-26 were unchanged and only total amounts were elevated. In SM-31 and SM-51, however, the sugar composition of fructose and glucose rations was elevated and total amounts were also increased. Matsuo et al. (1987b) reported that sugary and shrunken mutants showed higher total sugar and lower starch content, with water-soluble polysaccharides, in particular, being increased in sugary-type mutants. High sugar content related to mutants, i.e., *sugary*, *shrunken-1*, and *shrunken-2*, has been reported in rice (Yano et al. 1984). In this study, we identified a new high-sugar-type mutant that also contained high starch content, which contradicts the previously published report (Yano et al. 1984). This new mutant also showed high fructose and glucose contents. It was concluded that the mutation may have occurred at different loci than those reported previously (Yano et al. 1984).

Fatty acid analysis

Triglyceride is the major lipid found in rice grains, comprising glycerol attached by ester bonds to three fatty acids, includ-

Table 3. Content and composition of fatty acids in different endosperm type and grain mutants.

Number of mutant line	Character of endosperm	Composition of fatty acids [mg/g, (%)]					
		Palmitic acid (C16:1)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linoleni c acid (C18:3)	Total[1]
Wild type (Shindongjin)	Normal	7.36 (20.7)	0.56 (1.6)	11.84 (33.3)	15.18 (42.7)	0.58 (1.6)	35.51
SM-01(M)	Waxy	7.60 (22.7)	0.62 (1.9)	10.77 (32.2)	13.95 (41.7)	0.55 (1.6)	33.49
SM-04(γ)	Dull	9.94 (21.2)	0.88 (1.9)	15.79 (33.7)	19.53 (41.7)	0.74 (1.6)	46.87
SM-09(γ)	Chalky	8.58 (21.1)	0.74 (1.8)	13.72 (33.7)	16.94 (41.6)	0.73 (1.8)	40.71
SM-10(M)	Chalky	9.84 (19.1)	0.86 (1.7)	21.30 (41.3)	18.84 (36.5)	0.77 (1.5)	51.62
SM-21(γ)	Floury	8.94 (21.6)	0.59 (1.4)	14.15 (34.1)	16.66 (40.2)	1.11 (2.7)	41.46
SM-23(γ)	Floury (wrinkled)	13.41 (20.0)	1.13 (1.7)	25.26 (37.6)	26.28 (39.1)	1.08 (1.6)	67.17
SM-26(γ)	Shrunken	8.67 (22.9)	0.76 (2.0)	11.82 (31.2)	15.88 (42.0)	0.72 (1.9)	37.85
SM-31(γ)	Sugary	9.49 (18.7)	1.86 (3.7)	18.49 (36.3)	20.22 (39.7)	0.81 (1.6)	50.87
SM-32(γ)	Giant embryo	37.88 (30.4)	2.60 (2.1)	38.63 (31.0)	43.05 (34.60	2.40 (1.9)	124.56
SM-35(γ)	White core	11.29 (19.9)	1.00 (1.8)	20.08 (35.5)	23.36 (41.2)	0.91 (1.6)	56.64
SM-51(γ)	High Amylose	19.84 (22.1)	2.16 (2.4)	33.73 (37.6)	32.27 (36.0)	1.59 (1.8)	89.59
	Mean	13.15	1.58	24.34	25.41	0.96	66.65

(M): Lines induced by MNU.
(γ): Lines induced by γ-irradiation.
[1]: Total = palmitic acid + stearic acid + oleic acid + linoleic acid + linolenic acid.

ing oleic or linoleic acid. The fatty acid composition of rice is linoleic acid (50%), oleic acid (25-30%), and small amounts of saturated fatty acids, such as palmitic acid and stearic acid (Choudhury and Juliano 1980; Fujino 1978; Godber and Juliano 2003; Lee 1987). Lipids in the grain are eluted with amylose during cooking and confer a softness and tenderness to cooked rice. Lipid content in rice is considered as a desirable quality because unsaturated fatty acids, such as oleic, linoleic, and linolenic acids, could lower cholesterol levels in blood (Choudhury and Juliano 1980; Juliano 1985b).

The fatty acid content of selected endosperm and grain-shaped mutants is shown in Table 4. The fatty acid distribution in rice mutant samples was as follows; linoleic acid > oleic acid > palmitic acid > stearic acid > linolenic acid. Linoleic and oleic acids comprised higher proportion than all unsaturated fatty acids. Most mutants showed elevated fatty acid content and variations between mutants were detected. Relatively high fatty acids content were found in SM 32 (giant embryo-type mutant) and SM-51 (high-amylose-type mutant) at 124.56 and 89.59 mg/g, respectively. Common characteristics of those two mutants were crushed grains with a lower 1000-grain weight and narrower grain width. As a result, grains of these mutants should have a relatively thick aleurone layer.

The SM-32 (giant-embryo type) mutant contained 3-3.5 times as much linoleic acid and oleic acid and five times as much palmitic acid, stearic acid, and linolenic acid than the parental variety. Several giant-embryo mutants have been generated and reported (Koh et al. 1993; Matsuo et al. 1987a). A giant-embryo mutant had the same lipid composition as the wild type, with increased total lipid content by 36% (Matsuo et al. 1987a), while the others had 1.22-fold higher protein content, especially lysine, a restricted amino acid of rice grains, and 1.48- and 1.5-fold higher in total lipid content. In our study, SM-32 showed two-fold higher tocopherol content and four-fold higher unsaturated fatty acids content. Giant-embryo mutants contain more protein, lipid, vitamins, and plant sterols, which can reduce cholesterol and sugar levels in blood (Kang et al. 2003) and can have antioxidant and immunological activities (Kang et al. 2004a, b). The breeding objectives of rice can change over times depending on consumers' requests. Developing new rice varieties with improved quality will meet consumer demands. In this study, we developed new promising genotypes to meet such requirements. Trials should be further conducted on these genotypes to ensure their effective cultivation in the field. Among the mutants, the SM-32 line showed interesting and valuable characteristics that could be used for development of a high-quality functional food product.

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